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Evaluation of the Effect of Petrol Fumes on Calcium and Uric Acid Level of Petrol Attendants in Enugu State, Nigeria

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Abstract:
This work was carried out to evaluate the effect of petrol fumes on the calcium and uric acid concentration on petrol attendants in Enugu State, Nigeria. Total of seventy-nine (79) subjects were recruited voluntarily from some filling station (Total, NNPC, OANDO, JEZCO, AP, CONOIL) in Enugu state Nigeria. The study was made up of forty (40) petrol attendants and thirty-nine (39) subjects who are not petrol attendant (control). Blood sample were collected from all subjects who want to participate in the study and analyzed for calcium and uric acid concentration, using enzymatic spectrophotometric, O-Cresol Phthalin Complexone (O-CPC) method as described by Barnett et al., (1965), for calcium and uricase-POD enzymatic spectrophotometric method with 4-amino-antipyrine, described by Barhamet al., (1972), for uric acid. There was significant higher in mean concentration of uric acid (5.92 ± 4.61mg/dL) in petrol attendant individual in male and female when compared to the mean concentration of uric acid in male and female (3.91 ± 0.35mg/dL) in control group respectively, P<0.05 as shown in table 4.1 below. There was no significant higher in mean concentration of calcium (14.14 ± 2.83mg/dL) in petrol attendant individual in male and female when compared to the mean concentration of male and female control (14.64 ± 2.03mg/dL) group, p>0.05. But there is increased value of calcium level in prolonged petrol fumes exposure when compared with unexposed subjects. Prolonged exposure to petrol fumes can leads to increased hyperuricemia and hypercalcemia. Thus, exposure to petrol fumes may contribute to increase risk of calcium and uric acid related-diseases and disorders, like lung, liver, and kidney failure.

Keywords: *Calcium level, uric acid level, petrol fumes, petrol attendants*

1. Materials and Methods

1.1. Study Site
This study was carried out in some filling stations (TOTAL, NNPC, OANDO, JEZCO, AP, and CONOIL) in Enugu state, Nigeria. In some individual who are willing to participate in the study who has worked in filling station for a long time.

1.2. Subjects
The study comprises of a total of seventy-nine (79) subjects for calcium (aged between 20 and 40 years), consisting of 18 female control, 21 male control, 20 each for male and female petrol attendants. Uric acid 19 female control and 21 male control and 20 each petrol attendants respect to sex, and with known duration of exposure at different petrol stations in Enugu state, Nigeria. As (TEST), student of Madonna University, Elele Campus were selected as control, that is, that were not exposed to petrol fumes. The study and all information were kept confidential.

1.3. Consent
After explain the purpose of the reasrch to all subjects, their informed consent was obtained according to World Health organization (WHO) standards. Also before the commencement of the study, approval was obtained from the filling stations management.

1.4. Sample Collection
A 4ml of venous blood sample was collected from the median vein at the cubital vein in the fore arms of all the subjects who were in the sitting position for venepuncture which was carried out with minimal stasis, blood sample were then carefully transferred into a plain sterile container (no anticoagulant) and allowed to clot for 50 minutes then using applicator stick, the clotted blood was

dislodged and then centrifuged at 12,000rpm for five minutes. The serum obtained was carefully picked separated using an automatic micro-pipette and transferred into another plain specimen container and tightly screwed then it was analyzed immediately.

1.5. Reagents
Commercially prepared calcium and uric acid were obtained from spectrum diagnostic, Germany.

1.6. Biochemical Analysis
The following biochemical parameters were measured in order to study the effect of petrol fumes on the health status of petrol attendants:

1.7. Uric Acid Estimation
Uric acid level was estimated by enzymatic spectrophotometric method using **SPECTRUM** diagnostic kit (Germany) according to the procedure given in the kit protocol.

1.7.1. Method
uricase-POD enzymatic spectrophotometric method with 4-amino-antipyrine, described by Barham*et al*., (1972) was used to estimate serum uric acid concentration.
Assay principle: the assay is based upon the methods of modified trinderperoxidase assay using 3, 5-dichloro-2-hydroxybenzenesulfonic acid (DCHB), Trinder*et al*., (1972).
The series of the reaction involved in the assay system is as follows:

Uric acid is oxidized to allantoin by uricase with production of hydrogen peroxide

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{uricase}} \text{Allantoin} + Co_2 + H_2O_2$$

The peroxide react with 4-amino-antipyrine and (DCHB) in the presence of peroxidase to yield a quinoneimine dye.
The subchange in absorbance at 546nm is proportional to uric acid concentration in the sample.

$$H_2O_2 + \text{4-AAP} + \text{DCBH} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + H_2O$$

1.7.2. Procedure
Into clean 3 test tubes labeled test, standard and blank, 1.0ml each of reagent was pipette the test tubes, 20µl of sample was added into the tube labeled test and 20µl of standard regent is added into test tube labeled standard. The contents of the test tubes were mixed thoroughly and incubated for 5 minutes at 37^{o}C. The absorbance were obtained spectrophotometrically against the blank at 546nm staring with the least colored solution, zeroing with blank solution.

1.8. Determination of Serum Calcium Estimation
The **SPECTRUM** diagnostic kit (Germany) was used to measure the level of Calcium in the serum sample of both petrol attendants and control group.

1.8.1. Method
O-CresolPhthalin Complexone (O-CPC) as described by Barnett *et al*., (1965). As modified by the company that manufactured the reagent kit used Kessler *et al*., (1964).
Assay principle: calcium ions react with o-cresolphthalincomplexon (O-CPC) under alkaline conditions to form a violet colored complex. Magnesium and iron.

$$Ca^{2+} + \text{O-CPC} \xrightarrow{\text{Alkaline pH}} \text{calcium-O-CPC complex}$$

The color intensity of the complex formed is directly proportional to the calcium concentration. It is determined by measuring the increase in absorbance at 578nm.
Procedure: into 3 clean test tubes labeled test, standard, and blank, 0.5ml of reagent 1 and 0.5ml of reagent 2 was added to each of the test tubes, 10µl of sample was added into the test tube labeled test. 10µl of standard solution was added to the test tube labeled standard, reagent 1 and 2 was used as a blank by manufacturer's instruction. The content of the tubes were mixed thoroughly and incubated for 5 minutes at 20-25^{oC} .The absorbance were obtained spectrophotometrically against the blank at 578nm starting with the least colored solution, zeroing with blank.

2. Statistical Analysis
Data obtained were subjected to Statistical analysis using Statistical Package for Social Science (SPSS) version 20.0 Window 8.1 Results were expressed as mean ± Standard Deviation (S.D) while independent sample student's t- test and oneway Analysis of Variance (ANOVA) were used to test the difference between groups, values were considered significant at ($p<0.05$) and non-significant at $p>0.05$.

3. Results
There was no significant higher in mean concentration of calcium (14.14 ± 2.83mg/dL) in petrol attendant individual in male and female when compared to the mean concentration of male and female control (14.64 ± 2.03mg/dL) group, $p>0.05$.

There was significant higher in mean concentration of uric acid (5.92 ± 4.61mg/dL) in petrol attendant individual in male and female when compared to the mean concentration of uric acid in male and female (3.91 ± 0.35mg/dL) in control group respectively, P<0.05 as shown in table 1 below.

PARAMETERS	CONTROL	TEST	F-VALUE	P-VALUES
Calcium (mg/dL)	14.14 ± 2.83	14.64 ± 2.53	0.22	0.995
Uric acid (mg/dL)	3.91 ± 0.35	5.92 ± 4.61	9.256	0.000

Table 1: mean Calcium and uric acid concentration between petrol attendants (test) and non-petrol attendants (control)

3.1. Calcium and Uric Acid Concentration in Male (Control and Petrol Attendant) and Female (Control and Petrol Attendant) Individuals in Enugu State, Nigeria

As shown in table below there was no significant difference (p>0.05) in the mean calcium levels between the female control (14.76 ± 1.89mg/dL) compared to the female petrol attendants (14.15 ± 2.88mg/dL). Likewise, there was no significant difference (p>0.05) in the mean calcium levels between the male control (14.50 ± 3.17mg/dL) relative to the male petrol attendant (15.13 ± 2.77mg/dL). Overall, there was no significant difference in calcium levels between males and females.

Uric acid levels were significantly higher (p<0.05) in the male petrol attendants (7.19 ± 0.82mg/dL) compared to the male controls (4.67 ± 0.37mg/dL), female controls (3.36 ± 0.32mg/dL), and female petrol attendants (4.66 ± 0.43mg/dL). The uric acid levels were higher in the female petrol attendants (4.66 ± 0.43mg/dL) compared to the female control (3.36 ± 0.32mg/dL), although the mean difference was not statistically significant (p>0.05).

	Calcium conc. (mg/dL)	Uric acid conc. (mg/dL)
Female Control (FC)	14.76 ± 1.89^{a}	3.36 ± 0.32^{a}
Male Control (MC)	14.50 ± 3.17^{a}	4.67 ± 0.37^{a}
Female Test (FT)	14.15 ± 2.88^{a}	4.66 ± 0.43^{a}
Male Test (MT)	15.13 ± 2.77^{a}	7.19 ± 0.82^{bcd}
F- Value	0.022	9.256
P- Value	0.995	0.000

Table 2: Calcium and Uric acid concentration in male (control and petrol attendants) and female (control and petrol attendants) individuals in Enugu State, Nigeria

Values with different superscript on the same column are statistically significant at p<0.05. *Data represented as Mean ± SEM*

3.2. Calcium and Uric Acid Concentration in Individual Petrol Attendants at Different Duration of Exposure

As shown in table below the calcium levels were significantly higher (p<0.05) in the individual petrol attendant exposed for 21 – 30 months (27.17 ± 9.22mg/dL) compared to the control individuals (14.65 ± 1.84mg/dL) and those petrol attendants exposed for 0 – 10 months (11.67 ± 1.07mg/dL), 11- 20 months (10.83 ± 2.79mg/dL) and 31 – 40 months (14.71 ± 4.32mg/dL)

Uric acid levels were significantly higher (p<0.05) in the individual petrol attendants exposed for 0 – 10 months (5.81 ± 0.67mg/dL), 21- 30 months (6.17 ± 1.20mg/dL) and 31 – 40 months (6.68 ± 2.09mg/dL) compared to the control individuals (3.94 ± 0.26mg/dL).

	Calcium conc. (mg/dL)	Uric acid conc. (mg/dL)
Test	14.65 ± 1.84^{a}	3.94 ± 0.26^{a}
0-10 Months	11.67 ± 1.07^{a}	5.81 ± 0.67^{m}
11-20 Months	10.83 ± 2.79^{a}	5.47 ± 1.05^{a}
21-30 Months	27.17 ± 9.22^{bcd}	6.17 ± 1.20^{y}
31-40Months	14.71 ± 4.32^{a}	6.68 ± 2.09^{z}
F-Value	2.584	3.132
P- Value	0.044	0.019

Table 3: Calcium and uric acid concentration in individual petrol attendants at different duration of exposure

Values with different superscript on the same column are statistically significant at p<0.05. *Data represented as Mean ± SEM*

4. Discussion

Uric acid blood test also known as serum uric acid measures how much uric acid is present in the body (Mayo, 2007). The blood uric acid test measures the amount of the uric acid in the blood sample. Uric acid is produce from the natural breakdown of body's cells and from the food we eat. Most of uric acid is filtered out by the kidney and passes out of the body in urine. But if too much uric acid

is being produced from the breakdown of purine and kidney are unable to remove it from the body through urine, it will get to a point that the increase level of uric acid will start forming a solid crystal at the joint. This condition in a patient is called gout, it is a form of arthritis swelling in the joint, if remain untreated lead to forming hard lumpy deposit called tophi, high level of uric acid may also cause kidney failure, artherosclerosis, and diabetes (Kolz*et al*., 2009).

In these studies, uric acid and calcium concentration in subject exposed to petrol was evaluated. A significant increase in the mean uric acid was observed in petrol attendants ($p<0.05$) when compared with the control in the study.

There was no significant increase in the mean calcium concentration of petrol attendant ($p>0.05$) when compared with the control group. The work is not in agreement with the work done by Ayaz*et al.*, (2010), who said that there is increase in mean calcium level of automobile workshop the health status of auto mechanics in N.W.F.P Pakistan, the different in the result gotten may be due to other routine work exposure such as motor vehicle assembly, spray painting, burning of petrol, welding, brazing and repairing of radiators. This can contribute to an increase in mean calcium level of auto mechanics which petrol attendant are not expose with, but the result I got is in agreement with the work of Egwurugwu*et al*., (2014) who discovered an increase in human uric acid concentration and no increase in mean calcium level, but has increase effect on prolonged exposure to gas flares on human in Niger Delta region, Nigeria. The observed increase in uric acid level of the subject exposed to petrol. The aliphatic and aromatic hydrocarbon are major constituent of petrol, their metabolism can result to change in cell membrane due to reactive free radical species which are considered to be potential hapato and nephro toxicant in human affecting Calcium and uric acid level (Kristal-Bondy*et al*., 2006).

In vivo and in vitro studies suggest that lead-induced oxidation contribution to red blood cell and white blood cell damage (Yiin*et al.*, 1999). According to Bottefa*et al*., (1997) said that the major toxicant present in automobile workshop environment are polycystic hydrocarbon and Lead, best studied toxic effect of lead on heme synthesis (Habal, 2008). Lead can induce gout or artherosclerosis by inhibition of cytochrome p450 (Alvares*et al*., 1997) leading to an increase in serum uric acid following lead exposure (Skoczynska*et al*., 1993).

The observed increase in uric acid was likely due to lead induced inhibition of xanthineoxidase catalyzes the formation of uric acid from xanthine and hypoxanthine, which in turn produced from purine. Xanthineoxidase is a large enzyme whose action site consists of the metal molybdenum bound to sulfur and oxygen (Christensen *et al*., 2009). Within cells, xanthineoxidase can exist as xanthinedehydrogenase and xanthineoxidoriductase, which has also been purified from bovine milk and spleen extract Borghi*et al*., (2014) uric acid is released in hypoxic condition (Baillie *et al*., 2007). The increase observed in uric acid petrol attendant may result from the damage to hepatic cell by toxic pollutants (Eade and Henry, 2003). The observed decrease in calcium level in petrol attendant may have resulted from accumulation of calcium released in the plasma from the dying cells, tissues and membrane undergoing turnover (Ubani*et al*., 2010).

Also there was no significant difference in serum concentration of calcium level among the male petrol attendants and control when compared with the female petrol attendants and control ($P>0.05$) this is agreed with the finding of Bolland*et al*., (2010), who reported no increase in the mean level of kerosene and gas, due to accumulation of Calcium in petrol attendant released in the plasma from the dying cells, tissues and membrane undergoing turnover Ubani*et al*., (2010) caused by petrol fumes. But long period of petrol fumes exposure like two (2) years above can leads to increase in Calcium level as observed in table 4.3 above. The result agreed with the work of Toda *et al*., (1984) who reported increase in Calcium level of worker exposed to Cadmium oxide fumes after a long duration of exposure to the fumes

There was significant difference in uric acid concentration among the male petrol attendant and female attendants ($p<0.05$) when compared to male and female control.

Significant increase was observed in mean uric acid at different duration of exposure to petrol ($p<0.05$) when compared with their controls. This may be due to prolong exposure to constituent of petrol. The liver and kidney has a major role to play, in the liver where uric acid is synthesized while kidney removes execs uric acid from the body, therefore liver and kidney damage can lead to uric acid derangement with possible disturbance of cell membrane integrity which may cause excess uric acid to be released in the circulation, exposing an individual to a high risk of gout, polycythemia, diabetes, lymphoma, and multiple myeloma (Sanjay and Subir, 2005), uric acid is the best maker for the evaluation of gout (Kolz*et al*., 2009).

The result shown that exposure to petrol fumes may affect the uric acid level of petrol attendant, the result is in agreement with the finding reported by Dioka*et al*., (2004) the reported that occupational exposure of human subject to lead in petrol increase the concentration of uric acid (357±123µmol/L) in exposed subject compared with the unexposed subject. Similarly, Hernandez-Serrato*et al*., (2006) found that hyperuricemia was associated with blood lead concentration above 40microg/dL. Patil*et al*., (2007) has also reported significant increase in blood urea among battery manufacturing workers. The present study is also in correlation with study of Wang *et al*., (2002). It was found that there was significant increase in uric acid level among lead battery workers, it concluded that petrol fumes has adverse effect on the kidney function of petrol attendants workers.

5. Conclusion

The result showed that prolong exposure to petrol fumes may contribute to an increased uric acid level due to various toxic pollutant present in petrol, mostly the heavy metals exposing an individual to develop gout, renal dysfunction, artherosclerosis, diabetes, polycythemia, leukaemia and hypothyroidism. Therefore long duration of exposure to petrol fumes over two (2) years can increase Calcium level of those exposed to the fumes as shown in the table 4.3 above, which can cause hypercalcaemia, impaired kidney function and decreased absorption of other mineral. The increase that occurs in Calcium level of petrol attendant due to petrol fumes may be due to toxic pollutant and heavy metal like lead present in petrol. It concluded that petrol fumes has an adverse effect on the occupational health status of petrol attendants workers.

6. Recommendation

Before employment, it is important to screen the person(s) for history of certain condition to prevent exposing the individual at a high risk of petroleum distillate: also regular check on liver and kidney function of petrol attendants should be carried out to prevent toxic pollutant on uric acid and calcium related disorders. Also petrol pump attendant's dispensers should always wear protective or safety wears during work to avoid direct inhalation of the petrol fumes. Petrol attendants should use nose mask, protective clothing during work and good health education should be carried out by the primary health care board to sensitive the petrol pump attendants on how to develop the habit of using nose mask and protective clothing during work and also supply it to them. To enable them escape health challenges associated with petrol fumes pollution.

6.1. Aim

The aim of the study is to evaluate the effect of petrol fumes on calcium and uric acid concentration of petrol attendants.

7. Specific

7.1. Objectives of this Study Include

- To evaluate the calcium and uric acid concentration among petrol attendant at Enugu state, Nigeria.
- To evaluate the calcium and uric acid concentration among non petrol attendants to serves as control.
- To evaluate the calcium and uric acid concentration of Petrol attendant on different duration of exposure.
- To evaluate the calcium and uric acid concentration of male and female petrol attendant.
- To evaluate the effect of petrol fumes on calcium and uric acid level of petrol attendant and develop awareness for proper safety measures in this regard.

8. References

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